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The Clinical Significance of Unmineralized Bone Matrix in Man

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THE CLINICAL SIGNIFICANCE OF UNMINERALIZED BONE MATRIX IN MAN

by

Per-Erik Wiklund

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Abstract Bone biopsies were obtained from the iliac crests of 164 post-mortem cases and 252 patients referred owing to various clinical conditions. The biopsy technique of Burkhardt and the morphometric evaluation of Merz and Schenk were used, and the following variables were estimated: Trabecular bone volume, per cent trabecular surface covered with osteoid, per cent active osteoid - calcification fronts - and the number of osteoclasts in relation to the surface of the section. Using the bone samples from the post-mortem cases as normative values, the amount of osteoid - active and inactive - was studied in various diagnostic groups. The following conclusions could be drawn: 1. The Burkhardt instrument is a useful tool for obtaining good bone biopsies from the iliac crest. 2. The histological procedure and morphometric evaluation by Merz and Schenk are easy to handle once the method of hard tissue sectioning is mastered. 3. The working precision of estimation of osteoid is no better than 20 per cent but owing to the considerable inter-patient differences useful information can still be obtained. 4. Very little osteoid was found in individuals without any known or suspected metabolic bone disease - the normative data of the post-mortem cases in this study - considerably less than described by other investigators. It is most likely that deviations in the histological and morphometric techniques may explain this difference even if a real deviation may exist in the studied population. 5. Osteomalacia - increased amounts of osteoid - is rare in women with spinal osteoporosis. 6. The alkaline phosphatase which in women with osteoporosis is related to a low bone mineral content is unrelated to the amount of osteoid in these women. 7. Traces of impaired mineralization - osteoid seams in an increased abundance - were demonstrated in former concentration camp victims, possibly caused by a period of starvation in the past. 8. The amount of osteoid, in particular inactive osteoid, is increased in epileptic patients treated with anticonvulsant drugs. 9. Other findings in this group of patients are a noticeably high incidence of fractures and a low bone mineral content indicating that the finding of osteoid in such cases is evidence for the need of supplementary therapy. 10. In alcoholics there is no increase in the amount of osteoid, the mineralization of bone in these patients appearing to be undisturbed. 11. In alcoholics who also have undergone gastric resection, however, bone mineralization is disturbed as indicated by an increased amount of osteoid seams. 12. Although osteomalacia in its clinical and morphological sense is a rare condition, an increased amount of osteoid is a frequent finding in many groups of patients suspected of skeletal disorder. 13. On the other hand, in women with spinal osteoporosis without any other signs or symptoms of osteomalacia, increased osteoid is so rare that a bone biopsy is not justified.			
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by

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IN MEMORY OF MY FATHER

CONTENTS

	Page
INTRODUCTION	1
PAPERS	
Iliac crest biopsy in the diagnosis of metabolic bone disease. A method study (I)	7
Bone biopsy in women with spinal osteoporosis (II)	21
Alkaline phosphatase in women with osteoporosis (III)	23
Bone morphology in epileptics (IV)	27
Bone morphology in alcoholics (V)	33
GENERAL DISCUSSION	
Selection of methods	45
Normative data	46
Osteoporosis	47
Nutritional osteomalacia	47
Drug induced osteomalacia	48
Interpretations of increased osteoid	49
GENERAL SUMMARY AND CONCLUSIONS	51
REFERENCES	53

This thesis is based on the following papers:

- I. Wiklund, P-E.: ILIAC CREST BIOPSY IN THE DIAGNOSIS OF METABOLIC BONE DISEASE. A method study.
- II. Hulth, A.G., Nilsson, B.E., Westlin, N.E. and Wiklund, P-E.: BONE BIOPSY IN WOMEN WITH SPINAL OSTEOPOROSIS. Acta Med. Scand. 206: 205-206, 1979.
- III. Hulth, A.G., Nilsson, B.E., Westlin, N.E. and Wiklund, P-E.: ALKALINE PHOSPHATASE IN WOMEN WITH OSTEOPOROSIS. Acta Med. Scand. 206: 201-203, 1979.
- IV. Johnell, O., Nilsson, B.E., Wallöe, A. and Wiklund, P-E.: BONE MORPHOLOGY IN EPILEPTICS. Calcif, Tissue Int. 28, 93-97, 1979.
- V. Johnell, O., Nilsson, B.E. and Wiklund, P-E.: BONE MORPHOLOGY IN ALCOHOLICS.

These articles will be referred to by their Roman numerals in the text.

INTRODUCTION

Bone is composed of a ground substance containing glucosaminoglucones, protein and water with collagen fibres and calcium-phosphorus compounds - mostly crystalline - as stabilizing components.

In the course of bone formation a specific strain of cells, osteoblasts, with a high content of alkaline phosphatase, secrete a fibrous material - osteoid - which is composed of collagen (30 %), water (20 %), mineral (10 %) and other proteins (Herring 1972). After a brief period - 10 days - of maturation the osteoid is ready for mineralization and has by then assumed a width of 10 - 15 μm (Aaron 1976). During the mineralization process, normally lasting for 1 - 2 months, calcium-phosphorus compound crystals are deposited in the osteoid changing it into bone. The osteoblasts now tend to become trapped in the mineralized bone contributing the cellular element - the osteocytes.

If the normal sequence of events is being disturbed a condition with an increased amount of unmineralized osteoid - osteomalacia - may be the result.

Also, bone is constantly being removed, a process accomplished mainly by large multinuclear cells - the osteoclasts - usually located in well defined sites of erosion - resorption lacunae. Under normal conditions resorption and formation of bone are closely related and in balance - it has been proposed that, in addition to the influence of the parathyroid hormone, metabolites of vitamin D are engaged in both processes (Rasmussen and Bordier 1978).

Reports on deformities caused by softening of the bones are available from centuries back (Sherman 1950). It is not known when the term osteomalacia came into use but already in the 18th and 19th centuries the cause of osteomalacia was discussed, including its relationship with rickets. One generally agreed that these were two unrelated conditions until Pommer (1885) concluded that osteomalacia was a condition with areas of unmineralized bone and in general morphologically identical with rickets. Over the years single cases or small sets of cases of osteomalacia were reported who had been subjected to treatments varying from oophorectomy to prolonged administration of chloroform or autogenous vaccines. In recent years more extensive studies on larger groups of patients have been presented

such as those of Chalmers et al. (1969). In Scandinavia relatively few cases of clinical osteomalacia have been found and presented (Ask-Upmark 1937, Boström and Wester 1968, Boström et al. 1968).

In the thirties a group of Chinese workers (Hannon et al. 1934, Liu et al. 1935, Liu 1940) were able to clarify the relationship between osteomalacia, calcium intake, Vitamin D, kidney function and parathyroid hormone, suggesting that the primary defect is a lack of ability to absorb calcium from the intestine, a shortcoming which can only be remedied by vitamin D.

Albright et al. (1946) suggested that the term osteomalacia should only be used for such disorders in the adult as are associated with a presence of abnormal amounts of osteoid tissue and that such changes may be caused by any condition which depletes the calcium stores of the body. If the presence of osteoid tissue is accepted as a necessary requirement for the diagnosis of osteomalacia the diagnostic procedure must always include bone morphology.

Over the years it has been established that osteomalacia is the skeletal consequence of a variety of pathological conditions (Parfitt and Duncan 1975, Dent 1976). Such conditions are Vitamin D deficiency due to poor diet, too little sunshine or dark skin pigmentation, or Vitamin D malabsorption following gastric or small bowel resection or due to gluten enteropathy and other sprue syndromes including laxative abuse, and also following pancreatic and bile salt deficiencies. After the Vitamin D₃ 25-hydroxylation process had been further clarified, it has also been suggested that osteomalacia may be caused by liver conditions including alcoholism and by enzyme induction following the intake of anticonvulsant drugs. The impaired 1-hydroxylation process 25 OH D₃ to 1.25 (OH)₂ D₃ offers a possible explanation of the osteomalacia which is part of the osteodystrophy in chronic renal failure and maintenance hemodialysis (Stanbury 1972, Bordier et al. 1978, Rasmussen and Bordier 1978).

In addition a variety of conditions causing phosphate depletion and hypophosphatemia have been suggested as causes of osteomalacia. A rare disease is the primary familial Vitamin D-refractory osteomalacia (Falls et al. 1968, Stickler 1969). A common condition, hitherto not well documented, may be the osteomalacia caused by phosphate-binding aluminum-based antacides (Avioli 1979). Among other mechanisms, the

intake of large amounts of fluoride may also be mentioned as one cause of increased osteoid tissue. This phenomenon has been used in an attempt to treat osteoporosis (Jowsey et al. 1968).

In spite of all these possible etiologies, clinically apparent osteomalacia with skeletal pain and deformity, muscle weakness and radiological signs of demineralization such as Milkman fractures and Looser zones is rare. However, many investigators have adopted the hypothesis that osteomalacia in less obvious forms is fairly common in the population and particularly in the elderly population or at least in certain stigmatized groups. Andersson et al. (1966) and Chalmers et al. (1967) suggested that osteomalacia is a relatively common condition in elderly women and the latter author stated: "Treatment of osteomalacia is rapidly and consistently successful and well justifies a thorough screening of all elderly patients with weakness, skeletal pain, pathological fractures or with diminished radiographic density in bone." On the other hand Andersson et al. (1966) selecting from patients with symptoms of osteomalacia found morphological evidence of this condition in only 14 out of 100 patients.

Attempts of systematic search for osteomalacia in defined groups include studies on patients after gastric resection surgery (Morgan et al. 1965, Chalmers et al. 1967). In these studies, however, the incidence of osteomalacia, by its morphologic definition, was only a few per cent. Several other investigators have attempted to study the consequences on bone of gastric surgery but usually without presenting any histological evidence.

Also, groups of patients with fractures have been studied. Chalmers et al. (1969) found that about one fifth of patients with fracture of the upper end of the femur had increased osteoid. The same author in 1970 stated that subtrochanteric fracture of the femur was particularly closely related to osteomalacia and that such fractures should be followed by examination for this condition. Aaron et al. (1974) found in cases with fracture of the upper end of the femur, pathological amounts of osteoid in 20 per cent of the women and 40 per cent of the men.

Richens and Row (1970) conducted a study on residents of an institution for epileptics and found, purely basing their results on biochemical studies, an increased incidence of osteomalacia. Dent et al. (1970) demon-

strated severe osteomalacia in a few cases of epileptics on anti-convulsant therapy. However, the first systematic search for osteomalacia in epileptics was conducted by Mosekilde and Melsen (1976) who were able to confirm that epileptics in a high frequency had histomorphometric signs of osteomalacia indicated by the presence of unmineralized bone tissue.

Starvation has been suggested as a cause of osteomalacia. Dalyell and Chick (1921) and Hume and Nirenstein (1921) presented a large series of patients from First World War Vienna with a condition causing skeletal pain and muscle weakness which they referred to as hunger osteomalacia and successfully treated with cod liver oil. Maxwell (1935) found clinical evidence of osteomalacia -"adult rickets"- in a deprived population in China. However, no systematic histomorphometric study on this particular condition has been made.

Garner and Ball (1966) found in bone biopsies from mixed cases of malabsorption signs of osteomalacia in two thirds. Nordin (1961) found a coincidence between osteoporosis and steatorrhea and also, conversely, between steatorrhea and osteoporosis and, finally, between steatorrhea and osteomalacia. Atkinson et al. (1956) performed bone biopsies in a series of jaundice cases and found morphological osteomalacia in one third of the cases

The most fruitful systematic search for increased abundance of osteoid with the highest yield of obvious pathology has been in cases with renal failure n.b. uremia patients undergoing maintenance hemodialysis. The introduction of hemodialysis created a particular interest in the associated skeletal conditions (Stanbury 1972, Parfitt 1972, Hruska et al. 1978, Johnell et al. 1980, Denneberg et al. 1980).

Finally, search for osteomalacia has been conducted in patients with spinal osteoporosis. Lindahl (1960), screening women with osteoporosis with a calcium retention test, found pathological values indicating osteomalacia in one third of the cases. However, bone biopsies could not confirm this diagnosis and the author concluded that osteomalacia was indeed rare in osteoporosis patients. Schenk and Merz (1969) even found a slight decrease of osteoid in 26 patients with manifest senile osteoporosis. Johnson et al. (1971) found in iliac crest biopsies of osteoporotics an increasing amount of osteoid with age whereas in normal non-osteoporotic subjects this variable rather tended to decrease with age. Kruse and Kuhlencordt (1980) found 12.5 ± 7.8 per cent osteoid

surface in a group of osteoporotics which was considered normal compared with the normative data of Delling (1973).

One obvious difficulty in the studies on pathological conditions has been to define the normal values of unmineralized osteoid. Normative data have been presented by Sissons et al. 1967, Woods et al. 1968, Wakamatsu and Sissons 1969, Merz and Schenk 1970, Ellis and Peart 1972, Bordier and Tun Chot 1972, Meunier et al. 1975, Melsen et al. 1978 and others (Table 1).

The aim of the present study was to evaluate the usefulness of morphometric measurements of osteoid - uncalcified bone tissue - in women with osteoporosis, in patients with a known diagnosis such as epileptics on anticonvulsant drugs or severe misusers of alcohol and in an unselected series of patients referred for biopsy and morphometric evaluation under a variety of diagnoses.

Table 1. Comparison of normative data on osteoid, per cent of trabecular surface or per cent of total volume. (The pooled results of measurements in age- and sex-groups have been calculated. In several instances the data of other authors have been recalculated or derived from graphs.)

Source	Per cent surface			Per cent volume		
	AV	SD	Range	AV	SD	Range
Garner and Ball 1966				0.23		0 - 0.47
Sissons et al. 1967	12					
Woods et al. 1968	14		0 - 45	.1.1		0 - 5
Wakamatsu and Sissons 1969	6					
Chalmers et al. 1969	5		1 - 10			
Merz and Schenk 1970	16	9		0.51	0.33	
Bordier and Tun Chot 1972	17	5				
Ellis and Peart 1972				0.11	0.02	
Delling 1973			10 - 25			
Meunier et al. 1975	13	8				
Aaron 1976			2 - 24			
Melsen et al. 1978	17	7		2.1	1.4	
Hruska et al. 1978	13	14		2.0	2.3	
Present study (all available controls)	2.4	3.5		0.46	0.75	

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ILIAC CREST BIOPSY IN THE DIAGNOSIS OF METABOLIC BONE DISEASE:
A method study

by

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Österlund Foundations.

SUMMARY

The Burkhardt instrument is a useful tool for obtaining bone biopsies from the iliac crest in order to study bone tissue. Information on bone mass, however, is fairly unreliable and iliac crest biopsy has also a low predictive ability for spinal bone mass. Estimation of osteoid, however, is more useful and this variable cannot be studied by any other means. Even if the working precision is 20 % or even worse, the differences between patients are considerable and useful information can therefore be obtained. In an unselected group of patients, it was demonstrated that even if the number of cases which could be classified as osteomalacia was small, there were significant deviations in the amount of osteoid in many groups including seven Second World War concentration camp prisoners.

Key words: Biopsy, bone, osteoid, osteomalacia, osteoporosis.

INTRODUCTION

The iliac crest biopsy has proved to be of value in the diagnosis of metabolic bone disease especially after the introduction (1) of undecalcified sections which made it possible to estimate not only the bone mass but also the amount of mineralized and non-mineralized bone and the proportion of other measurable variables such as resorption and formation surfaces.

Various techniques have been introduced concerning the biopsy sites and instruments used as well as the histological methods and morphometric evaluations (1-9). The reproducibility of iliac crest biopsy values and the accuracy of the iliac crest as a site for evaluation of the human skeleton have been studied by Beck and Nordin (3), Vost (10), Bordier et al. (4), Garner and Ball (5), Dunnill et al. (11), Ellis and Peart (12), Melsen et al. (13).

The objective of the present study was to examine further the method of iliac crest biopsy, in particular the biopsy technique described by Burkhardt (6), the morphometric technique by Merz and Schenk (9) and the usefulness of biopsy for the diagnosis in a variety of clinical conditions.

METHODS

Biopsy technique

The biopsy technique and the instrument of Burkhardt (6) were originally intended to be used in hematological diagnostic work but has later been adopted by workers in the field of metabolic bone disease.

The operation is comparatively simple, being performed with the patient in the supine position and under surgically sterile conditions and local anesthesia. Through a small skin incision over the left iliac crest, approximately 3 cm behind the anterior superior iliac spine, the funnel of the Burkhardt instrument is inserted and pressed into the periosteum of the almost flat surface of the crest (Figures 1-2).

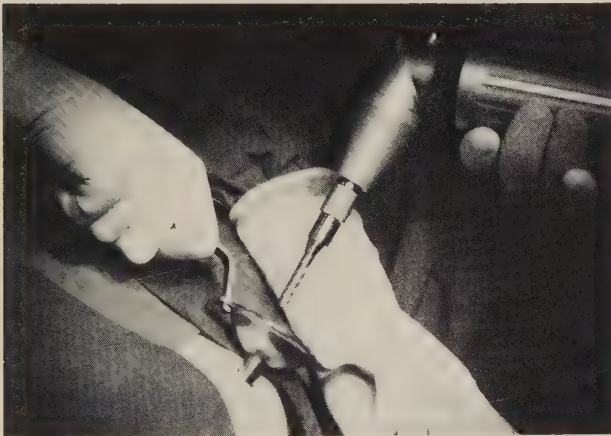


Figure 1. The Burkhardt funnel-shaped retractor is inserted and pressed against the periosteal surface of the iliac crest.

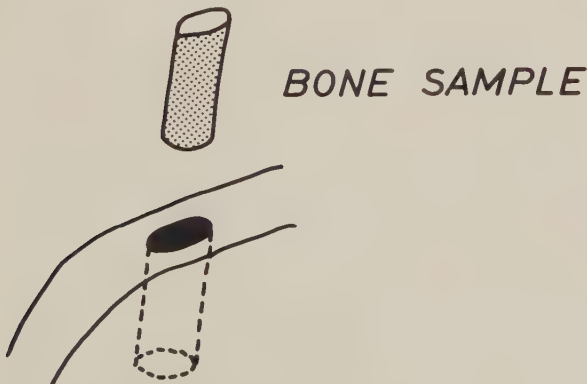


Figure 2. Biopsy site.

The entire procedure is performed through this narrow funnel shaft which retracts the skin and subcutaneous tissue and also restricts the depth of the biopsy.

The bone sample is obtained with a slow-moving motor-driven miller and is cylindrical with a diameter of 0.5 cm and a length of 1.5 cm. Owing to the low speed of the drill, there is very little contusion or deformation of the trabecular structures of the cancellous bone. The bone defect is packed with sterile oxidized cellulose tissue^{x)} in order to prevent post-operative bleeding, and the skin being closed by 1-2 sutures.

Histological method

The bone samples are fixed in 10 per cent buffered formalin for at least 24 hours and then dehydrated with alcohol in increasing concentrations (40, 70, 96 and 99.9 per cent) during another 24 hours for each concentration. After that, defatting is carried out in a 3/1 solution of 99.9 per cent alcohol and chloroform for 24 hours, followed by rinsing in xylol. The bone samples are now ready for embedding in methyl-metacrylate with an addition of benzyl-peroxide and plastoid N in 37° for 48 hours.

The samples are then sectioned in a hard sectioning microtome longitudinally making sure that the sections for morphometric evaluation are collected from the central part of the specimen. For the evaluation of bone volume and osteoid surface 5 μm sections are prepared and stained according to Goldner's (14) modification of the Masson trichrome staining. For the estimation of calcification fronts, 10 μm sections are prepared and stained with toluidine blue.

Morphometric evaluation

For the morphometric evaluation a template consisting of a point-wave pattern in the eye piece of the microscope (9), an objective magnification of 13 x and an eye-piece magnification of 10 x are used. With this magnification, thirty fields can be measured in most sections including only trabecular bone. From the Goldner-stained sections the total bone volume, and the osteoid surface as per cent of trabecular bone surface are calculated and furthermore the number of osteoclasts per mm^2 of the sample surface is measured over a surface of 50 mm^2 , using an objective magnification of 40 x and an eye-piece magnification of 10 x (15). In the

x) Surgicel^R, Ethicon Ltd

toluidine blue-stained sections, an evaluation of the calcification fronts is made and expressed as per cent of osteoid surface (Figure 3).

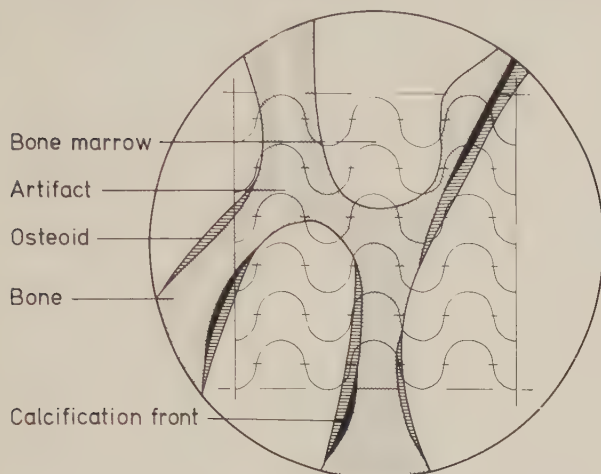


Figure 3. Schematic representation of morphometric variables in trabecular bone.

MATERIAL

1. Method study

This study was made in 25 random autopsies of patients who had died in the Malmö General Hospital. No attempts were made to exclude cases with gastro-intestinal or metabolic bone disease. From each of these 25 cases, two biopsies were obtained from the left iliac crest 3 and 6 cm behind the anterior superior iliac spine and one biopsy from the right iliac crest 3 cm behind the anterior superior iliac spine, all with the Burkhardt instrument. From the central part of the fourth lumbar vertebra, a 1 x 1 x 2 cm bone sample was collected with a chisel.

The bone specimens were treated with the histological methods described above. The amount of trabecular bone and percentage of trabecular bone surface covered with osteoid were calculated. The values from the four biopsy sites were compared.

The reproducibility of the morphometric method was tested by double estimations of the left iliac crest samples one year apart and by the same observer. To test the reproducibility of the osteoid estimate,

however, iliac crest biopsies from 20 patients on maintenance hemodialysis who had obvious histological signs of osteomalacia were used. These biopsies were measured by the same observer on two different occasions with several months interval.

In an effort to evaluate the risks of the biopsy procedure, the records of 412 patients who had undergone an iliac crest biopsy were searched retrospectively for complications such as post-operative hematoma, wound infection or other unwanted effects.

2. Normative data

Iliac crest samples for the control series were obtained in the Department of Forensic Medicine from autopsies of cases of sudden death due to myocardial infarction, suicide or trauma, i.e. mostly traffic accidents. Cases with known renal or hepatic disease and with signs of alcoholism or gastro-intestinal surgery were excluded.

A total of 139 iliac crest biopsies were collected, 67 from women and 72 from men. The sex and age distribution of the sample is shown in Figure 4.

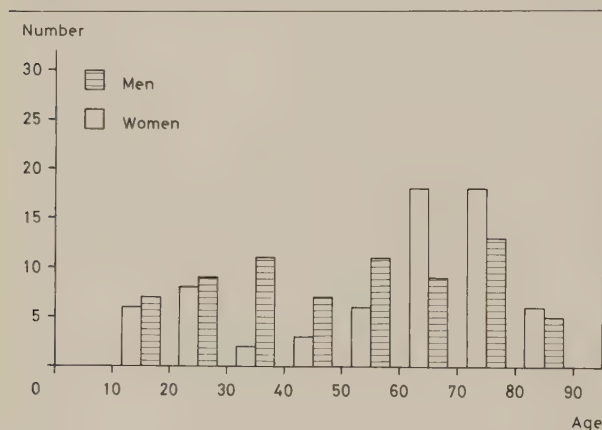


Figure 4. Age and sex distribution of normative data.

The specimens were sectioned and evaluated morphometrically in structures corresponding to the Burkhardt biopsy. The data obtained from these cases - trabecular bone volume, per cent trabecular bone surface covered with osteoid, per cent osteoid with calcification fronts and osteoclast activity - have been used as reference values.

3. Clinical cases

Iliac crest biopsy was performed in 127 patients; the age and sex distribution is presented in Figure 5. The cases for biopsy were chosen in several departments in the hospital by a number of physicians and for a variety of reasons.

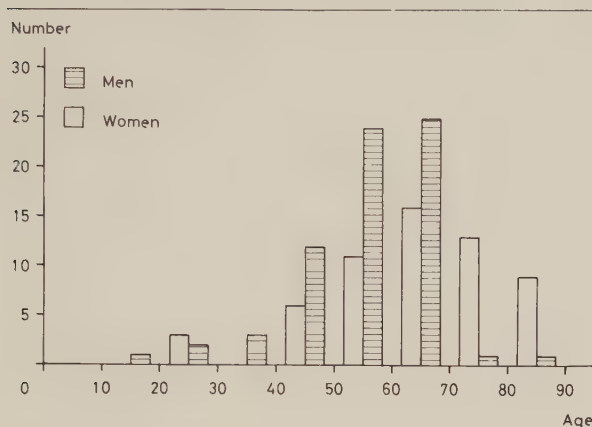


Figure 5. Age and sex distribution of clinical cases.

The clinical series may be subdivided in the following groups according to the basic condition causing the biopsy:

Gastric resection, multiple fractures, back pain (combined with other diagnoses or back pain only), alcoholism and miscellaneous signs or symptoms of malabsorption or other metabolic disease that could influence the skeleton. In the latter group there were seven former prisoners from German concentration camps.

The values for trabecular bone volume and the per cent of trabecular bone surface covered with osteoid were calculated and in the cases where osteoid covered more than 5 % of the trabecular bone surface, an evaluation of calcification fronts was made and expressed as the per cent of osteoid surface.

RESULTS

Complications

Out of 412 patients, 22 (5 %) developed some kind of post-operative complication. Fifteen had a hematoma, four superficial wound infections and three interference with the lateral cutaneous nerve of the thigh. Out of the 15 hematoma cases only two were such that a few days of hospitalization

were required whereas the remainder only caused symptoms during a brief period of time. Most of the hematoma cases were seen during the first year after introduction of the biopsies. Two of the three cases with signs of nerve injury recovered completely within 2 months whereas the third patient two years after the biopsy, had still decreased superficial sensation in the lateral aspect of the thigh.

Method error

In Table 1, the errors involved in the assessment of bone mass are presented. The repeated counting of one single section presents an error of 7 % whereas the in-between sample error in the same patient is about 30 %.

Table 1. Errors of bone volume assessment (N = 25)

Source	Coeff. of variation
Counting one section twice	7 %
Comparing front-back samples	31 %
Comparing left-right samples	27 %

The ability of an iliac crest to represent other parts of the skeleton n.b. the spine is presented in Table 2. Whether a single sample or an average of samples from the iliac crest is used the correlation is highly significant but still with a fairly low predictive value since only about half of the total variation is accounted for.

Table 2. Correlation between biopsy sites - bone volume

Source	Coeff. of correlation
Front left iliac crest vs L 4	0.66
All three iliac crest samples vs L 4	0.72

} $p < 0.001$

Table 3 shows the error of estimating osteoid surface. When a completely new section of the same sample is counted the error is doubled.

Table 3. Errors of osteoid surface assessment (N = 20)

Source	Coeff. of variation
Counting one section twice	10 %
Counting two sections in the same sample	20 %

Osteoid

Excess osteoid seams were found in most subsets of the biopsy group regardless of the primary cause of the biopsy (Table 4).

Table 4. Osteoid, per cent of trabecular surface - comparison between controls and subsets of biopsy cases (note, each patient may be included in more than one group).

Group	N	Average	SD	Number >12 %	Difference from controls
Controls	85	2.4	3.5	-	-
Gastric resection	24	5.0	4.4	2	p<0.01
Multiple fractures	36	4.5	4.9	4	p<0.01
Back pain	101	3.7	4.2	6	p<0.05
Back pain only	38	2.8	3.1	1	n.s.
Alcoholics	28	2.8	3.6	1	n.s.
Miscellaneous	24	5.5	6.3	4	p<0.01
Concentration camp prisoners only	7	6.3	8.0	1	p<0.02

The large standard deviations indicate skewedness of the data as has been demonstrated in the controls (16). A group of patients who did not deviate significantly from the controls were patients with back pain only, without any known associated condition such as partial gastrectomy, alcoholism or multiple fractures. Similarly, the alcoholics did not deviate significantly from the controls with regard to the abundance of osteoid. The greatest numerical deviation from the controls was demonstrated in the former concentration camp prisoners. Table 4 also shows the number of cases with an osteomalacia according to our definition (osteoid surface >12 % - computed as 95 % confidence limits on the basis of the normative data). In those patients who had, in the Goldner-stained sections, osteoid in excess of 5 %, the calcification fronts were measured and the relationship between total osteoid and active osteoid (calcification-covered osteoid) is presented in Figure 6. An increased amount of osteoid is associated with a relative decrease in the amount of active osteoid - osteomalacia. The patients with increased osteoid were found in most groups and could not be distinguished from their calcification fronts.

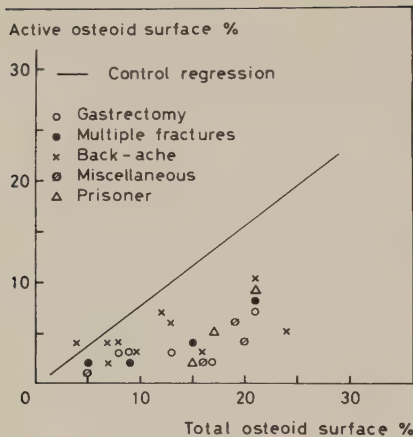


Figure 6. The relationship of active and inactive osteoid in biopsy cases.

DISCUSSION

The biopsy of Burkhardt appears to be one of the best techniques for this particular purpose. The biopsy can be carried out in outpatients, under local anesthesia and as long as proper attention is given to the method of infiltration of anesthetics, the site of the biopsy and the packing of the biopsy defect, the procedure should cause little discomfort to the patients and hardly any complications at all. Woods et al. (8) and Beck and Nordin (3) also reported very few or no complications with iliac crest biopsy. An additional advantage with the Burkhardt biopsy is that the geography of the bone is left intact, fractures in the sample being seen only in some patients with severe osteoporosis.

Even if the evaluation of the bone mass or volume in a single section can be carried out with relative precision, it is obvious that the site of the biopsy is crucial. Probably, even if the counting precision within a single sample is infinitely improved, the between-sample or between-site error will amount to 20 or even 25 %. Similar data have been presented by Ellis and Peart (12) and Garner and Ball (5). Bordier et al. (4), however, claim that with a very thorough study of each biopsy, an inter-biopsy error of 10 % may be attained in transiliacal samples including both cortices. Beck and Nordin (3) failed to find any systematic difference between biopsy sites along the iliac crest. Melsen et al. (13) found a variation of 15 - 20 % in samples taken from the iliac bone but in that study also the deeper structures of the bone and not only the iliac crest

were included. Visser et al. (17) proposed that the reliability of measuring surface parameters of bone depends on the cutting direction and of the measuring method and that the representativeness in relation to the whole skeleton of such data remains obscure. Vost (10), using a photometric method of bone-volume determination, described a poor relationship between the iliac crest and the L IV. Also, Dunnill et al. (11) suggested that the bone mass of a vertebral body cannot be predicted from measurements of the iliac crest. In the present study it appears as if only half of the total variation in lumbar spine bone content is related to the iliac crest measurement. It is therefore not possible to make predictions of spinal bone mass from iliac crest biopsies in an individual case whereas in group studies the variable may be useful. However, the accuracy of iliac crest bone morphometry as a measure of spinal bone mass or total body bone mass is probably less than that of forearm gamma-absorptiometry (18). Also, Lindahl and Lindgren (19) from a study on human autopsy samples concluded that small bone specimens were of a doubtful value for estimating the degree of osteoporosis in single individuals. Given these circumstances the non-invasive absorptiometry method should be preferred for evaluation of bone mass in patients. However, in the evaluation of bone biopsies, the bone volume variable and also the trabecular bone surface variable have to be measured and are important corrective factors for other morphometric variables in the same section.

To estimate the osteoid surface only one sample was available for each of the hemodialysis patients. The error, 10 % for measuring one sample, was of the same order of magnitude as found by Melsen et al. (13) and less than the approximately 30 % which can be computed from the data of Woods et al. (8). Melsen et al. (13) also found comparatively small variations in osteoid surface between various parts of the iliac bone. The working precision of our method is probably not better than 20 % and even worse in patients with only small changes. However, since the interpatient and the inter-group variations are large the method serves its purpose.

It has been suggested that osteomalacia is a fairly common condition particularly in the elderly (20). The main purpose of our biopsies was to disclose cases of osteomalacia or, rather, cases with an increased abundance of osteoid. In a study of osteoporotics, however, we failed to demonstrate any significant increase in osteoid (16). In the present study of primarily unselected cases we could, nevertheless, demonstrate

a small but significant increase in most groups even if the diagnosis of osteomalacia could rarely be secured. Except for patients with alcoholism or back pain without associated disease as the primary cause of biopsy, there was a moderate increase in most groups of the amount of osteoid. Particular interest is attached to the seven individuals who had spent part of the Second World War in concentration camps. After liberation they were taken to Sweden, arriving in an emaciated and generally miserable condition. At the time of the biopsy there were no objective signs of malnutrition and the time interval between the liberation from concentration camp and the biopsy was approximately 30 years. Nevertheless, there was an increased amount of osteoid to be seen in the biopsies of most of these individuals. It is generally assumed that starvation causes osteomalacia in adults. Hitherto, no morphological studies on concentration camp victims have been recorded.

It appears as if measurements of osteoid may be useful in at least some of the groups included in the study, such as patients who had undergone gastric resection, patients with multiple fractures and patients with miscellaneous metabolic disorders. For the further evaluation of osteoid we have used the calcification fronts in order to estimate the activity of the osteoid seams. Bordier and Tun Chot (21) and others have considered a low calcifying activity of the osteoid seams as a prerequisite for the diagnosis of osteomalacia. This concept has not been entirely unchallenged. Woods et al. (22) suggested that the ratio calcification front/osteoid surface has no significance as an indicator of osteomalacia, but is merely an expression of cellular activity which may be modified by several factors. Regardless of the actual interpretation of a decreased abundance of calcification fronts, this decrease is an almost constant finding in a variety of conditions usually associated with osteomalacia such as renal osteodystrophy (Johnell et al.: In preparation), epilepsy (23) and as in the present study.

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Bone Biopsy in Women with Spinal Osteoporosis

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ABSTRACT. Iliac crest biopsies were obtained from 110 women, 62 of whom had been clinically and radiologically classified as osteoporotics. The amount of osteoid surface was measured by a histological planimetric method. It was demonstrated that the amount of osteoid does not differ between osteoporotic women and control women and that osteomalacia is rare in both groups.

Key words: bone biopsy, osteomalacia, osteoporosis.
Acta Med Scand 206: 205, 1979.

A constant feature in idiopathic osteoporosis is the absence of pathological laboratory findings. Nevertheless, it may be assumed that a group of women with signs of osteoporosis such as back-ache and radiological changes contains one or more subsets in which further diagnostic work is profitable. Among others, Andersson et al. (2) and Chalmers et al. (4) formulate a hypothesis according to which osteomalacia is a fairly frequent condition in the elderly possibly because of malnutrition, and particularly in women. Osteomalacia is in marginal cases a difficult diagnosis. The most reliable tool is probably the bone biopsy (5).

The purpose of the present study was to test the usefulness of iliac crest biopsy for the diagnosis of osteomalacia in a group of women classified as osteoporotics.

PATIENTS

Osteoporotics

Sixty-two consecutive women were selected depending on clinical symptoms and radiological signs of spinal osteoporosis. All patients had been first referred because of back-ache. On the initial radiogram the diagnosis of osteoporosis was based on an obviously low radiological density, crush-fractures or both. None of the patients had a history of severe dietary deficiency, malabsorption or renal disease. Their alkaline phosphatase levels were within normal limits.

Controls

Iliac crest samples were obtained from 48 autopsies on women in the Department of Forensic Medicine. Included were individuals who had suffered sudden death, mostly cardiacs or death by violence. Excluded were individuals who were known to be alcoholics or who had undergone gastrointestinal surgery. The age distribution of both samples is demonstrated in Fig. 1.

METHODS

Estimation of osteoid

The method and instrument of Burkhardt (3) were used. In local anaesthesia a small incision was made over the iliac crest and a cylindric 0.5–1.5 cm bone sample was obtained with a motor-driven miller. This is the method to collect a sample without deforming the cancellous bone.

The undecalcified samples were imbedded in methyl-metacrylate and 4–6 μ m sections were made in a hard-section microtome. The sections were stained by a modified Goldner (6) method. In the resulting slides the osteoid tissue can be distinguished from the calcified material by colour. The surface occupied by bone and osteoid was measured by counting intersections between bone, osteoid and a wave pattern engraved on the eye-piece of the microscope (7). Only trabecular bone was evaluated. The abundance of osteoid tissue was calculated as the percentage osteoid surface of total bone surface.

RESULTS

The per cent osteoid was 3.2 ± 4.0 and 2.7 ± 4.0 (average $\pm$ S.D.) in osteoporotics and controls, respectively. There was no significant difference between the groups. As indicated by the standard deviations, the variable is skewedly distributed (Fig. 2). There was no correlation between age and per cent osteoid in this study.

DISCUSSION

The skewedness of the data would imply that in osteoporotic women the bone contains no or almost no detectable osteoid whereas in some indi-

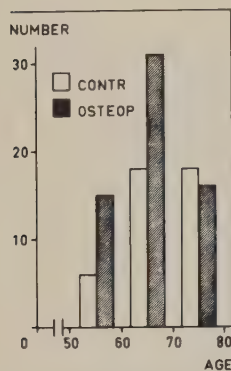


Fig. 1. Age distribution of osteoporotics and controls.

viduals the amount of osteoid is slightly increased because of some skeletal disorder. However, the same distribution was found in the controls. Values below 10% cannot be referred to as pathological. This should be compared with the findings of Aaron (1) who proposed that the normal upper limit for osteoid in an English population is 24% of the trabecular surface. Only in one of the 62 osteoporotics a definite increase above normal could be demonstrated. She was a woman whose symptoms did not differ from the others, nor did she have any abnormalities in serum calcium, serum phosphorus or alkaline phosphatase in repeated measurements.

Our conclusion is that bone biopsy as a routine screening method to detect osteomalacia in women with osteoporosis does not return the efforts invested. It should be limited only to patients with the signs and symptoms deviating from the usual pattern of osteoporosis.

Most of the data indicating that osteomalacia is common in old women has been collected in Britain. Possibly, nutritional habits and other ethnical particularities contribute to the discrepancies. Our observations and conclusions are therefore valid only under the present conditions in Southern Sweden.

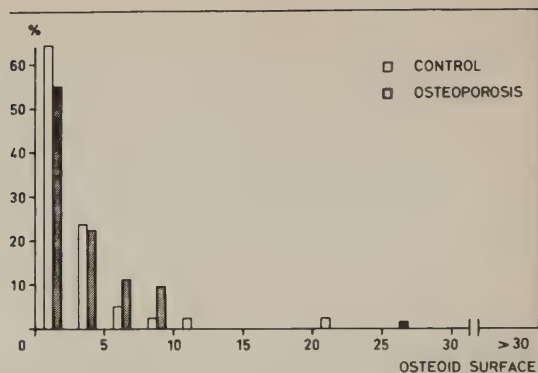


Fig. 2. Distribution of osteoid in osteoporotics and controls.

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Alkaline Phosphatase in Women with Osteoporosis

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ABSTRACT. Altogether 15 partially independent measurements of bone mass in 100 women with clinical and roentgenological signs of osteoporosis were correlated to the alkaline phosphatase activities of the same individuals. There was a slight but significant negative correlation indicating an increasing alkaline phosphatase activity with decreasing bone mass. This correlation was not caused by interaction of age. There was no correlation between alkaline phosphatase activity and clinical or morphological signs of osteomalacia. The changes could not be explained by fractures. It is suggested that a slight increase in the alkaline phosphatase activity in women with a more severe osteoporosis is related to bone resorption.

Key words: alkaline phosphatase, osteomalacia, osteoporosis.

Acta Med Scand 206: 201, 1979.

The serum alkaline phosphatase is frequently used in clinical diagnostic work as a screening test of bone disease mainly for the disclosure of osteomalacia or for studies on the consequences for skeletal metabolism of renal disease and hemodialysis.

The purpose of this study was to relate this variable, alkaline phosphatase, to parameters of bone mass and composition in a series of women with clinical signs of osteoporosis.

SELECTION OF PATIENTS

For the study were selected 100 women who had sought medical advice because of back-ache and who had radiological evidence of spinal osteoporosis. Clinically, there was a large variation with cases ranging from doubtfully pathological to severe osteoporosis with deformity of the spinal column and multiple fractures. The women were all post-menopausal, their age at the time of the investigation was 63.3 ± 7.6 years. Their menopausal age had been 46.7 ± 4.5 (mean $\pm$ S.D.).

METHODS

Alkaline phosphatase

The alkaline phosphatase was estimated by a modified Busch and Busch method, using 2-amino-2-methyl-1-propanol buffer, para-nitro-phenyl-phosphate as substrate and magnesium as activator with an optimal pH of 10.8. With this method the normal variation in adults is 2–8 units within 95% confidence limits.

Spinal osteoporosis

Radiological density was classified 0–4 with increasing severity of radiological osteoporosis visible on lateral radiograms of the lumbar spine (11).

The number of crush fractures, 0–5 or more, was determined from lateral radiograms of the thoracic and lumbar spine.

The vertebral ratio was calculated as the ratio between the central height and the sagittal diameter of the body of the lumbar vertebra located most closely to the central beam in lateral radiograms of the lumbar spine. Only in 73 cases the radiograms were of sufficient quality for this measurement.

The spinal ratio is defined as the ratio between the height of the head plus trunk, measured in sitting position, and the total height. This variable is a parameter of spinal shortening (8).

Iliac crest biopsy

A 0.5×1.5 centimeter cylindric bone sample was obtained from the left iliac crest by the method of Burkhardt (2). The un-decalcified sample was imbedded in methylmetacrylate, sectioned and stained by a modified Goldner technique. The surface and the volume of bone and osteoid in the sections were estimated using a point and wave pattern engraved on the eye-piece of the microscope (6). Also, the osteoid was calculated and expressed as % of total bone. Sixty-four biopsies of sufficient quality to be included were obtained.

Gamma absorptiometry

The bone mass in the forearm was measured by a photon absorption technique (7). The method is similar to that of Cameron and Sorensson (3), the main difference being the source of radiation, americium-241 instead of iodine-125. The bone mass was measured 1 and 6 centimeters proximally of the dorsal distal edge of the ulna.

Table I. *The relationship between alkaline phosphatase and parameters of bone mass*

Variable	No.	r	p
Spine			
Radiological density	99	0.21	$0.05 < p < 0.02$
Crush fractures	98	0.15	$p > 0.1$
Vertebral ratio	73	-0.10	$p > 0.1$
Spinal ratio	87	-0.12	$p > 0.1$
Iliac crest			
Bone surface	64	-0.26	$0.05 > p > 0.02$
Bone volume	64	-0.17	$p < 0.1$
Forearm			
Left 1 cm	93	-0.33	$0.01 < p < 0.001$
Right 1 cm	96	-0.24	$0.02 < p < 0.01$
Left 6 cm	94	-0.25	$0.02 < p < 0.01$
Right 6 cm	90	-0.33	$0.01 < p < 0.001$
Metacarpus			
Left	97	-0.22	$0.05 < p < 0.02$
Right	97	-0.17	$0.1 < p < 0.05$
Femur			
Left	99	-0.22	$0.05 < p < 0.02$
Right	99	-0.27	$0.01 < p < 0.001$
Trabecular pattern	95	-0.18	$0.1 < p < 0.05$

Metacarpal and femoral cortices

The cortical thickness was measured on the midshaft of the second metacarpal and the femur in anteroposterior radiograms. In both instances the combined cortical thickness was measured and corrected for the width of the bone (1, 4).

Femur trabecular pattern

The trabecular pattern of the proximal end of the femur was estimated on anteroposterior radiograms of the hip according to the method of Singh et al. (12). Both hips were used for the estimate.

Statistics

The relationship between alkaline phosphatase and the various parameters of bone mass was studied by the method of linear correlation. It should be taken into account that some of the variables, radiological density, number of crush fractures and trabecular pattern are non-parametric.

RESULTS

The alkaline phosphatase for the entire group was 6.7 ± 2.0 units. There was no correlation between age and alkaline phosphatase ($r=0.04$, $p>0.1$). In 9 out of 15 parameters of bone mass there were significant correlations indicating increasing alkaline phosphatase activity with decreasing bone mass (Table I). In some of the variables there were suggestive relationships with the same tendency in all of the correlations, significant or not, the tendency was homogeneous. (Note that radiological

density and number of crush fractures will increase in rank with decreasing bone mass.)

There was no correlation, whatsoever, between the amount of osteoid, total or relative to bone mass, and alkaline phosphatase (Table II).

Twelve women had alkaline phosphatase activities of 10 or more (10–14 units). Their records were searched for possible distinguishing trends. In no case did the bone biopsy show osteomalacia. In one instance the alkaline phosphatase was probably measured within a few weeks after a vertebral compression fracture. The activity became normal later on. In another case the elevated value was found shortly after the bone biopsy was taken. In the remaining cases there was no clinical information suggesting a close relationship in time with fracture. In most instances more than one value over normal limits was recorded. The 12 women did not deviate in age from the average of the sample, nor appeared there any clinical features distinguishing this group.

Table II. *The relationship between alkaline phosphatase and osteoid*

Variable	No.	r	p
Osteoid surface	64	0.00	$p > 0.1$
Osteoid volume	64	0.00	$p > 0.1$
% osteoid surface	64	0.03	$p > 0.1$
% osteoid volume	64	-0.03	$p > 0.1$

DISCUSSION

The alkaline phosphatase activity of the women in this study was increased only slightly above the normal average. Only a minority of the patients had definitely abnormal values.

The object of the test was to identify patients with metabolic bone disease other than idiopathic osteoporosis, particularly osteomalacia. Clinically, this was not successful, and the alkaline phosphatase activity was not related to the amount of osteoid tissue found in bone biopsies.

Roberts (10) and Keating et al. (5) demonstrated that the alkaline phosphatase activity increased with age in healthy women. In the present study including only women with clinical osteoporosis and with a more narrow age range no such correlation could be demonstrated. Therefore, the inverse relationship between bone mass and alkaline phosphatase activity is probably not a result of interaction of age.

Nilsson and Westlin (9) demonstrated that in women with femoral neck fracture the alkaline phosphatase activity was elevated during the first two months after the injury. No such relationship with fracture could be demonstrated in the present study.

From the correlation coefficients it can be seen that there is a considerable residual variance in the alkaline phosphatase data, less than 10% of the variance is explained by correlation to bone mass.

The relationship between alkaline phosphatase and reduction of bone mass found in this study has not been previously described. The reason for this relationship is not known but it is suggested that also in idiopathic osteoporosis an increased alkaline phosphatase is a sign of increased bone resorption.

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Clinical Investigations

Bone Morphology in Epileptics

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Summary. In 31 epileptics, most of whom had been on anticonvulsive drugs for decades, the amount of osteoid—active and inactive—and the osteoclast activity were measured in iliac crest biopsies and compared with the same variables from a control group. Although falling within normal limits, the amount of osteoid, in particular the inactive osteoid, was significantly increased in the epileptics. The osteoclast activity was also significantly increased in the epileptics.

Key words: Osteomalacia — Anticonvulsant drugs — Epileptics — Calcification front — Osteoclasts.

Introduction

Osteomalacia in epileptics on anticonvulsant drugs has been reported by several investigators. The diagnosis has been based on blood chemistry, radiography, and quantitative bone mineral measurement [1–6]. The bone morphology in epileptics has been studied in two cases by Dent et al. [7] and in 60 cases by Mosekilde and Melsen [8].

We have found six times the expected number of non-seizure-related fractures in a group of epileptics over a 7-year period [9]. In most of these epileptics we have also studied the bone mineral content which was reduced by 10–15% (Lidgren et al., in preparation). In addition, patients were found who had changes in biochemical variables suggestive of osteomalacia or hyperparathyroidism [10].

The objective of the present study was to relate these findings to bone morphology with special reference to osteomalacia.

Material

Institutionalized Epileptics

Included in the study were 22 epileptics, 9 men and 13 women, age 56 ± 12 (mean $\pm$ SD). They were all admitted to an institution (Fogdaröshemmet) mainly on grounds of mild mental retardation. The cases were selected for this study to represent those epileptics among a group of 71 who had a history of fracture indicating bone fragility and/or had abnormal blood and/or urine chemistry (Table 1). Also, the selection was limited to those epileptics who were considered able to give informed consent to the biopsy procedure. Most of these epileptics had suffered from convulsive disease since childhood and all had been on anticonvulsant drug therapy for many years. The median time of treatment was 37 years. Thirteen patients were receiving diphenylhydantoin (100–500 mg) and twelve phenobarbital (100 mg). Carbamazepine was used in 5 patients and primidone in 4. Three epileptics had diphenylhydantoin as the only drug. In one epileptic the treatment had been stopped 4 years previous to the biopsy, but he had in the past had medication for at least 30 years. None was receiving vitamin D, calcium supplement, or steroid therapy. None had diabetes or rheumatoid arthritis. As reported earlier [10] 13 epileptics had a combination of an increased alkaline phosphatase and a reduced urine calcium indicating osteomalacia. Biopsies were obtained in nine of these.

Noninstitutionalized Epileptics

Biopsies were also obtained from 9 epileptics treated as outpatients, 5 men and 4 women, age 33 ± 10 . These epileptics had been treated with diphenylhydantoin, except for one man who had been treated with a combination of primidone and phenobarbital. The treatment had been continuous for many years. The median time of treatment was 20 years. All these epileptics were working, none had had fractures, and there was no history of kidney disease, diabetes, steroid therapy, or gastric surgery. One epileptic had had epidemic hepatitis 30 years previously; otherwise, there was no history of liver disease in these cases. One of the epileptics had increased serum alkaline phosphatase; there were no other abnormal biochemical findings.

Controls

Control samples were obtained from 85 autopsy cases from the Department of Forensic Medicine who had suffered sudden

Send offprint requests to Bo Nilsson at the above address.

Table 1. Abnormal biochemical variables in institutionalized epileptics [22] selected for biopsy

Biochemical variables	No. of epileptics
Increased serum Ca (> 2.6 mmol/l)	4
Increased alkaline phosphatase (> 4.6 μ -kat/l)	11
Decreased urine calcium ($< \begin{cases} 288 \\ 198 \end{cases}$ mmol/mol creatinine)	13
Fragility fractures without seizure	7 (15 fractures)

death by traffic accident, suicide, stroke, or cardiac arrest and who had no known history of gastric surgery or alcoholism. The age of the controls was 54 ± 21 .

Methods

A 0.5×1.5 cm cylindrical sample was obtained from the left iliac crest with a motor-driven miller [11]. The samples were embedded in methylmethacrylate and 5μ m thick longitudinal sections were obtained. The sections were stained according to the method of Goldner [12]. The volume of bone, the surface of the bone trabeculae, and the surface covered by osteoid seams were calculated using a point-wave template according to Merz and Schenk [13]. In each biopsy two whole sections were measured, usually 30 template fields.

The active osteoid surface (in this study defined as the osteoid surface with calcification fronts) was measured in separate sections from the same biopsy. These sections were stained with toluidine blue and the total and the active osteoid were measured with the same point-wave template. The

osteoid surface was the same measured in the Goldner- and the toluidine-stained sections although there was an unsystematic intrapatient variation. Only subjects with an osteoid surface exceeding 5% measured in the Goldner-stained samples were measured in toluidine blue staining since the variable—active osteoid—could not be estimated with any precision in subjects with less total osteoid surface. Thus 19 controls and 16 epileptics were included in this part of the study.

The abundance of osteoclasts was evaluated by counting the number of osteoclasts per square millimeter of section surface. Osteoclasts were defined as multinuclear cells located in immediate conjunction with a trabecular bone surface [14].

Results

The osteoid-covered trabecular surface was significantly greater in the institutionalized epileptics than in the control cases ($P < 0.001$) (Fig. 1, Table 2). The osteoid surface was not age or sex related.

In the separate study on active osteoid using the toluidine technique, a significant ($P < 0.001$) difference was found between epileptics and controls in the composition of the osteoid (Fig. 2). In both groups there was a fairly constant relationship between active osteoid and total osteoid, but in the epileptics only $37 \pm 11\%$ of the osteoid was active as compared with $73 \pm 17\%$ in the controls. In the nine biopsies from the epileptics with biochemical suspected osteomalacia, the osteoid seams covered $11 \pm 5\%$ of the trabecular surface as compared with $5 \pm 3\%$ in the remaining biopsies, a significant difference ($0.01 > P > 0.001$). However, osteoclast activity and the ratio active/inactive osteoid did not deviate from those in other epileptics.

The number of osteoclasts was significantly greater in the institutionalized epileptics than in the controls ($P < 0.001$) (Fig. 3, Table 2). Since there was no sex difference in the two variables, the data from the men and the women were pooled. The nine noninstitutionalized epileptics differed in both respects from the normal controls but not from the

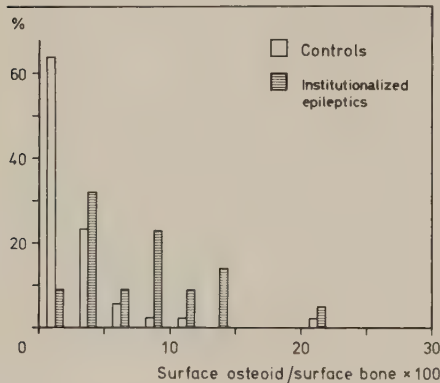


Fig. 1. Distribution of osteoid surface in institutionalized epileptics as compared with controls

Table 2. Osteoid-covered trabecular surface and abundance of osteoclasts in controls and epileptics^a

Group	Osteoid, % surface	Osteoclasts (per mm ²)
Controls (N = 85)	2.4 ± 3.5	0.04 ± 0.02
Institutionalized epileptics (N = 22)	7.5 ± 4.9	0.10 ± 0.06
Noninstitutionalized epileptics (N = 9)	7.6 ± 8.0	0.10 ± 0.03

^a Data are recorded as mean ± SD

institutionalized epileptics (Table 2). In the control sample the number of osteoclasts decreased significantly with increasing age. However, when this variable was corrected for the total amount of bone in the sample, there was no age regression and also the corrected values differed between epileptics—institutionalized and noninstitutionalized—and controls (analysis of covariance). In the 22 institutionalized epileptics the number of osteoclasts, the percentage of osteoid-covered trabeculae, the serum alkaline phosphatase, the serum calcium, the serum phosphate, and the urine calcium were tested for correlation. There was a significant positive correlation between the serum phosphate and the osteoclast count ($0.05 > P > 0.02$). Otherwise, there were no significant correlations. The osteoclast count in the four slightly hypercalcemic patients did not deviate from normal.

There were no differences in the morphometric variables among the various types of medications, nor could the duration of the medication be demonstrated to influence the pattern.

Discussion

Osteomalacia

In the past it has been assumed that the loss of bone mineral so frequently encountered in conjunction with anticonvulsant drug therapy [4, 6] is due to osteomalacia. However, histologic confirmation of this assumption had been somewhat doubtful. Dent et al. [7] presented signs of osteomalacia in biopsies from two patients. Mosekilde and Melsen [8] found an increased amount of osteoid in comparatively young adult outpatients with epilepsy on anticonvulsant drugs. The method of evaluation of osteoid may differ and differences may exist among ethnical groups. Aaron [15] proposed that the normal upper limit for osteoid was 24% of the trabecular surface. High values for the upper normal limit of osteoid have also been presented by Schenk et al.

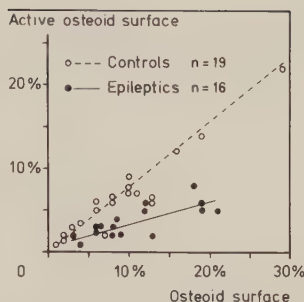


Fig. 2. The relationship between active osteoid and total osteoid in epileptics and controls. (Regression equation for controls: $y = 0.7x - 0.1$, $r = 0.96$; for epileptics: $y = 0.3x + 0.8$, $r = 0.79$)

[16] using the same method as in the present study, whereas lower normal values were presented by Jowsey et al. [17]. In the present study the average for normal was only a few percent, the distribution of the data being obviously skewed. Probably only values exceeding 10% should be considered abnormal (Fig. 1). Therefore it is difficult to establish whether the morphologic changes in the bone of epileptics should be defined as osteomalacia. On the contrary, it seems as if the epileptics form a subset of the population with a slightly higher than average content of osteoid tissue which is still, however, within reasonable limits.

In one aspect, however, despite the low values of total osteoid, the diagnosis of osteomalacia in at

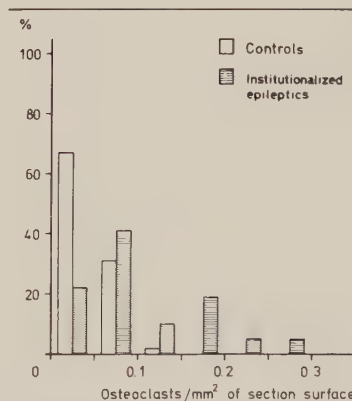


Fig. 3. Distribution of osteoclasts in institutionalized epileptics as compared with those in controls

least some of these epileptics is supported by the findings of a changed relationship between active and inactive osteoid. This has been pointed out as a special feature of anticonvulsant osteomalacia [18].

Osteoporosis

The findings of Christiansen et al. [4] and Hahn et al. [6] clearly demonstrate that there is a loss of bone mineral in epileptics following therapy with anticonvulsant drugs. This loss has been confirmed in a series of institutionalized epileptics (Lidgren et al., in preparation). In measurements of the relative volume of trabecular bone in the iliac crest, Mosekilde and Melsen [8] failed to demonstrate any difference between normal controls and young epileptics in either sex. Similarly, a reduction of bone volume in epileptics which seemed apparent in the present study was not significant. However, iliac crest biopsy is an insensitive method for detection of small changes in bone mass (Hulth et al., in preparation). Rödbro et al. [19] could demonstrate in the early phase of anticonvulsive medication to epileptics a loss of bone mineral, whereas Lidgren et al. (in preparation) found after decades of medication a reduction which did not much exceed that reported by Rödbro et al. [19]. In both studies the loss of bone mineral is sufficient to cause an increase in the risk of fractures [20].

Osteoclastic Resorption

Mosekilde and Melsen [8] measured osteoclastic resorption from the percentage of trabecular bone surface occupied by resorption lacunae and the mean volume of pericytic lacunae. There was an increase of about 50% in both variables in young epileptics as compared with healthy controls. In the present study this variable was evaluated from a count of osteoclasts. The difference between epileptics and controls was of the same magnitude or greater in our cases in that the osteoclast count was more than doubled in the epileptics. The positive relationship between osteoclast abundance and serum phosphate remains unexplained, but does not indicate an increased parathyroid activity.

Pathogenesis of Bone Disease in Epileptics

Bone fragility with fracture of the upper end of the femur, the upper end of the humerus, and the distal end of the forearm is a sign of osteoporosis rather than osteomalacia. Also, the loss of bone mineral

found in a previous study of the same set of epileptics indicates a loss compatible with an increased fracture incidence. The morphological findings in iliac crest biopsies, however, indicate a pathology not compatible with osteoporosis, namely, an increased abundance of osteoclasts and a slightly, but significantly, increased amount of osteoid tissue, in particular inactive osteoid—osteoid without calcification fronts. These findings, which were present in old inactive as well as in younger epileptics who led an otherwise normal life, are extensive enough to indicate osteomalacia, increased parathyroid activity, or both. When compared with the data of previous studies dealing with bone pathology caused by anticonvulsant drugs in an early stage of the treatment, our findings indicate that the condition is to some extent self-limiting in that decades of treatment do not precipitate more severe morphological changes but rather a steady state is attained.

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BONE MORPHOLOGY IN ALCOHOLICS

by

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SUMMARY

The osteoclast activity- number of osteoclasts per surface bone section - and the osteoid abundance - percentage of trabecular bone surface covered with osteoid - was measured in iliac crest biopsies from 38 alcoholics, all men. These alcoholics had a history of decades of over-consumption of alcohol and of traumatic and medical complications due to their abuse. Also the bone mineral content of the forearms was decreased at least in the older of the alcoholics so that their pattern of bone mineral content in relation to age was similar to that of women rather than to men.

The osteoid was increased in alcoholics but only in those who had also undergone gastric resection in the past whereas in the remainder there was no difference in osteoid between alcoholics and control data of patients without known alcoholism or known bone disease. Osteoclasts, however, were more abundant in the iliac crest biopsies from alcoholics than from the control group. The deviation was greater in alcoholics who had also undergone gastric resection although in the remaining cases there was also a significant increase above normal. The findings indicate that the nutritional effects of alcohol misuse on the skeleton were far from serious since no signs of osteomalacia developed, whereas the daily drinking in itself may precipitate changes, hormonal or other, with an inherent ability to induce bone resorption.

Key words: Alcohol, osteoporosis, osteomalacia, osteoclast, bone mineral.

Saville (1) found in bone samples from the iliac crest of departed alcoholics that the bone ash content of the middle aged alcoholic man was of the same order of magnitude as that of an old woman. In vivo studies in alcoholics (2-3) confirmed that the bone mineral content has indeed decreased in this group of patients. In addition, it has been demonstrated that alcoholics have fractures more often, particularly the types of fracture that we tend to relate to bone fragility (4-7). The alcoholics are an elusive group of patients. It is difficult to establish with any certainty the degree and the duration of the misuse, and even the definition of alcoholism may be discussed at length. Furthermore, diagnostic work is in many instances impossible to carry out seeing that the patients decline hospital admissions and are unable to keep appointments.

In the Department of Orthopedic Surgery of the Malmö General Hospital

a bone biopsy service has been offered over the years which provides iliac crest bone biopsy with the Burkhardt (8) instrument and morphometric evaluation of the bone samples. In a production control study of this activity it was found that not less than 38 patients without doubt qualified as alcoholics. In this group, the bone biopsies had been obtained over a period of 5 years. Although the data on the patients are afflicted by the scantiness specific for this group, and despite the fact that the study is retrospective, we have felt justified in publishing the series since there is no similar data available in the literature.

The objective of this presentation was to compare some morphometric variables of bone in alcoholics with normative data obtained from individuals without known bone disease.

MATERIAL AND METHODS

Patients

Included in this study were 38 patients age 50 ± 9 years, all men, with multiple fractures, back ache or skeletal pain arousing suspicion of skeletal disorder. All patients qualified as alcoholics by filling at least one of the following criteria:

Repeated bouts over the years with concomitant injuries, emergency room consultations and roentgen examinations.

Repeated admissions to institutions for alcoholics.

Liver cirrhosis from alcohol misuse.

Delirium tremens.

So far as can be established all these patients all had had an abnormal alcohol intake for 10 years and usually twice as long. Also, they were all "active alcoholics" who had so far been unable to change their drinking habits.

In the record room at the Department of Diagnostic Radiology - the only department with an emergency roentgen service in the city of Malmö - the records of the 38 patients were searched for trauma episodes over the last 25 years. As compared with age-matched controls the number of trauma episodes was overwhelmingly increased in the 38 alcoholics (Table 1).

Table 1 Information obtained on alcoholics (N = 38) and controls without known misuse of alcohol (N = 38) from the records 1950 - 1976 in the Department of Diagnostic Radiology.

	Skull roentgen	Fragility fractures	Other fractures	Negative roentgen- examinations	Medical complications
Alcoholics	67	29	73	91	24
Controls	2	3	13	19	0

Thirteen of the patients had undergone gastric resection, the Billroth II procedure, 16 ± 10 years prior to their bone biopsy.

Bone mineral measurement

Twenty-seven of the patients also volunteered for measurement of bone mineral content of the forearms in close relation to the bone biopsy. The method of gamma-absorption-metry as modified by Naucière et al. (9) was used Gambro Bone Mineral Detector. The measuring device consists of a radio-nuclide (Americium-241) source which moves, synchronized with a scintillation detector across the forearm measuring the bone mineral in the radius and the ulna 6 cm proximal to the tip of the ulnar styloid process. Except for inpatients with previous injuries to the upper limb, both forearms were measured and the average calculated. For this series normative data obtained from 140 men without injury to the upper limbs or known bone disease were used for comparison (10).

Bone biopsy

Bone biopsy was performed under local anesthesia using the instrument of Burkhardt (8). A low speed motor driven cylindric miller is inserted through a small incision over the iliac crest. A 0.5 x 1.5 cm cylindric sample is removed.

The morphometric procedure

The un-decalcified samples were embedded in methyl-metacrylate and the 5 μ thick longitudinal sections were obtained and stained according to the method of Goldner (11). The volume of bone, the surface of bone trabecula and the surface covered by osteoid seams were calculated using a point-wave template according to Merz and Schenk (12). One section from the thickest part of the sample was measured which usually permitted

the evaluation of 30 template fields. Only trabecular bone was included. For the purpose of studying active - calcifying - osteoid seams those samples in which the osteoid surface according to the Goldner technique was in excess of 5 % of the total trabecular surface were sectioned again and stained with toluidine blue. The osteoid-covered trabecular surface was measured once more and in addition the dark border lines between osteoid and bone which with this staining indicate calcification. Finally, again in the Goldner stained sections, the osteoclasts were evaluated by counting the number of osteoclast cells - multinuclear cells located in immediate conjunction with the trabecular bone surfaces. The whole sample was searched and the abundance of osteoclasts expressed as number of cells per mm^2 of total section surface. Also this variable could be corrected for the amount of bone in the sample by calculating the number of osteoclasts per bone section surface.

The morphometric variables were compared with data on 85 autopsy cases from the Department of Forensic Medicine. These cases had suffered sudden death by traffic accident, suicide, stroke or coronaries and had no known history of gastric surgery or alcoholism. For the comparison with the alcoholics, only the male controls were used and a sample was drawn which gave an approximately age-matched control group.

RESULTS

The bone mineral content was significantly ($p < 0.01$) decreased in the alcoholics as compared with age-matched controls (Figure 1). The rate of loss with age appears to be slightly more rapid in those alcoholics who had also undergone gastric surgery ($p < 0.05$). This subset of patients was slightly older than the average alcoholic in our group (54 ± 9), but even after correction for age there was a significant difference. Alcoholics with more than two fractures over the years had a lower bone mineral content than the average alcoholic but were also older, and after correction for age there was no difference.

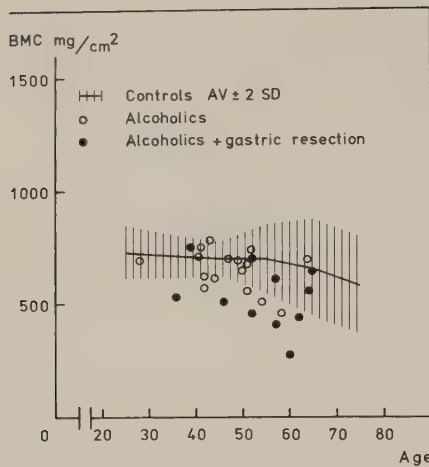


Figure 1. The relationship of age and bone mineral content in alcoholics and controls.

The osteoid surface was somewhat greater in the alcoholics than in the controls. In Figure 2 the total set of control cases is compared with the alcoholics. Also, the difference was tested statistically between alcoholics and approximately age matched control men (Table 2, T-test of skewed one-tailed distributions). The alcoholics deviated significantly from the controls in that the amount of osteoid was greater. However, after removing the 13 patients with gastric resection the difference was no longer significant.

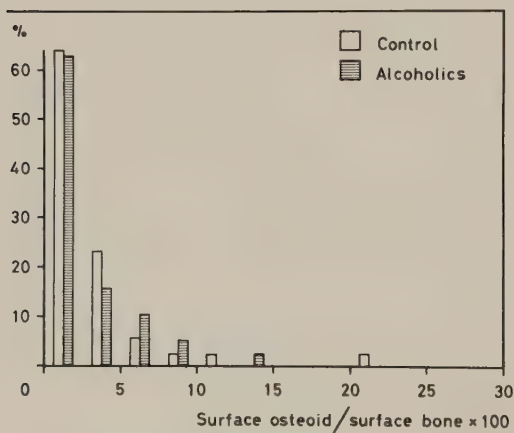


Figure 2. The distribution of osteoid in alcoholics and controls.

Table 2. Osteoid surface (per cent of total trabecular surface) in alcoholics and controls.

	N	Av	SD	Deviation from controls
Controls	51	1.4	2.1	
Alcoholics				
Gastric resection	13	4.6	4.1	$p < 0.001$
Non-gastric resection	25	2.1	3.2	n.s.

The relationship between active and inactive osteoid is demonstrated in Figure 3. The alcoholics have a reduced amount of active osteoid per unit osteoid tissue indicating a low calcification activity. The rate is just under 30 % of active osteoid as compared with about 70 % in the controls. The difference is highly significant ($p < 0.001$). However, most of the cases included in this part of the study, those with a substantial amount of osteoid - more than 5 % in the Goldner-stained section- had also undergone gastric surgery. Nevertheless, the two patients without gastric surgery in the past also showed the same tendency of a low portion of active osteoid.

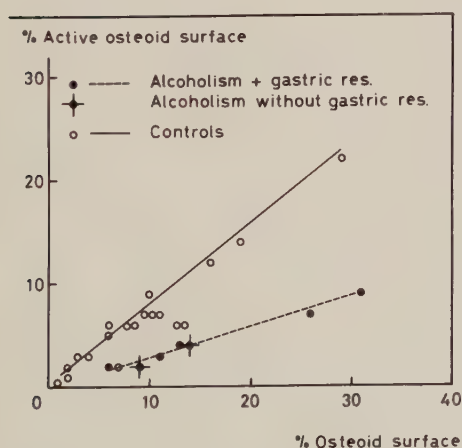


Figure 3. The relationship between active osteoid and total osteoid in alcoholics and controls, only patients with $> 5\%$ osteoid in the Golder stained sections are included.

The osteoclast activity was increased (Figure 4) in the alcoholics with gastric resection ($p < 0.001$) as well as in alcoholics without ($p < 0.01$). Between the two groups - alcoholics with and without gastric surgery - there was also a significant difference ($p < 0.02$) (Table 3).

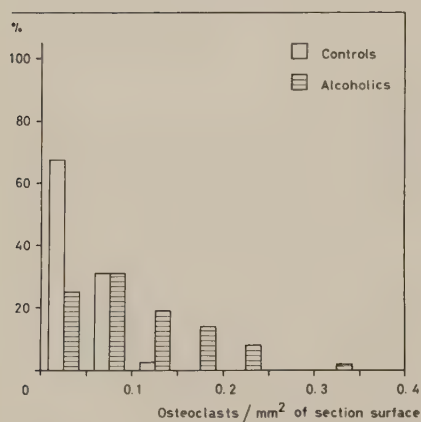


Figure 4. Distribution of osteoclast count in alcoholics and controls.

Table 3. Osteoclast count in alcoholics and controls

	per mm ² total section surface			per mm ² bone section surface		
	N	Av	SD	N	Av	SD
Controls	35	0.04	0.02	35	0.3	0.1
Alcoholics						
Gastric resection	11	0.14	0.08	11	1.2	0.9
Non-gastric resection	25	0.08	0.06	25	0.6	0.5

DISCUSSION

The high frequency of gastric resection in these patients was probably - to some extent- due to the selection process. However, Kristensson et al. (7) were able to demonstrate that every third male alcoholic in

Malmö had a roentgen verified peptic ulcer when the history of the patient had been reviewed a few decades back. Also, the alcoholic ulcer patient is more difficult to manage with conservative treatment and, probably, much more often develops bleeding complications forcing gastric surgery to be undertaken. Even if today the Billroth II-procedure has lost its popularity and been replaced by other methods, gastric resection is still in some instances indicated in conjunction with bleeding episodes and will therefore probably be a continuing complication in alcohol misuse. The finding of marginal osteomalacia in patients who have undergone gastric surgery is not new. So far, the longest series consists of 85 patients who after partial gastrectomy had raised serum alkaline phosphatase, low serum calcium or bone pain. Only six of these patients had osteomalacia whereas about 1/4 of the remaining patients had more than the normal proportion of trabecular surface of the bone covered with osteoid, a finding similar to that of the present study (13). It is, however, somewhat unexpected that osteoid seams are still so abundant in spite of the fact that the gastric resection took place on average 16 years prior to the biopsy. In those 25 patients who had no previous gastric surgery there was no clear-cut case of osteomalacia and the increase in osteoid was not significant. This implies that nutritional deprivation is of little importance for their loss of bone. It may be that the alcoholics in our series, however qualified, do not represent the worst cases, namely those who do not seek medical attention until forced to do so in the final act of their misuse and who die young. Nevertheless, our patients represent the type of problem that we encounter in our daily practical work. Also, several died of their misuse within a few years.

Osteoclast activity, a sign of bone resorption and increased parathyroid activity, is probably a fundamental function in the process of bone loss with disease and age. However, in osteoporotic women increased osteoclast activity is extremely rare and this group does not deviate from other individuals of the same age and sex (14). In this aspect the alcoholics are different from other osteoporotics in that their osteoclastic activity is raised above average indicating an ongoing bone resorption. At present, the reason for this is unknown. However, Kalbfleisch et al. (15) showed that alcohol intake causes a prompt magnesium and calcium diuresis, a finding which has been verified at a later date. In normal men alcohol rapidly increases the PTH level (16). Also, alcohol causes profound stimulation of adrenal cortex in rodents (17). Finally, decreased physical activity induced by alcohol consumption is related to the particularly low bone mineral contents in some alcoholics (3). Whatever the process, it produces in male

alcoholics a bone mineral pattern similar to that of women (Figure 5), thus inducing a female fracture pattern in the alcoholic male which is superimposed on the fractures and other injuries directly related to episodes of intoxication. It is more than unlikely that this pattern could be broken by any other means than reduction of the alcohol intake.

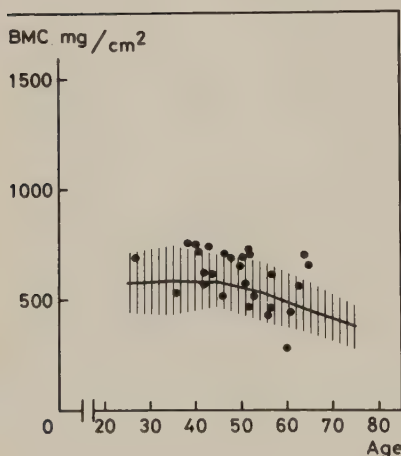


Figure 5. The bone mineral content of male alcoholics plotted on the data of female controls.

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GENERAL DISCUSSION

Selection of methods (I)

Our biopsy method and our method for histo-morphometric analysis were originally intended to meet a demand for a diagnostic procedure with a potential of diagnosing osteomalacia in patients with a variety of known or suspected skeletal conditions referred to our department. In addition, methods have later been developed to study the cellular elements of bone (Johnell 1980). The original method chosen, the vertical biopsy of the iliac crest using the Burkhardt (1966) biopsy instrument, and the morphometric evaluation using the template of Merz and Schenk (1970), had the advantage of being established and fairly simple. The Burkhardt iliac crest biopsy can be easily carried out under local anesthesia in out-patients and has very few complications if any. The Merz and Schenk morphometric technique is simple, once the method of hard tissue sectioning is mastered. Later developments in biopsy techniques, in particular the transverse iliac crest biopsies suggested by Bordier and Tun Chot (1972), have not, in our opinion, been of sufficient importance to induce any change in our methods in the course of the data collection process. Changes of methods will probably not improve the poor precision which is the major difficulty in this context as long as multiple biopsies or very large biopsies cannot be obtained. However, in the course of the data collection process, one additional method was introduced which required re-sectioning and toluidine blue staining of a great number of samples. Adams et al. (1967), Bordier and Tun Chot (1972) proposed that the part of the osteoid which in the toluidine blue staining comes out as dark blue or black granules in the osteoid-bone interface - the sites of active calcification or calcification fronts - must be taken into account and that this active osteoid should be distinguished from inactive. Increased osteoid and decreased calcification fronts should be the criterion of osteomalacia. Also, Aaron (1976) suggested that a decrease in calcification fronts should be a requirement for the diagnosis of osteomalacia. A similar method of distinguishing active and inactive osteoid is the solochrome cyanin staining method suggested by Meunier (1975). Another approach to the problem of defining active mineralization sites is the use of tetracycline labeling (Frost 1969 and Mosekilde and Melsen 1980).

Since the precision of evaluation of osteoid in normal or nearly normal individuals is very poor, a lower limit of 5 per cent osteoid

surface was set, expressed as the per cent of total trabecular surface, and only cases with osteoid in excess of that were studied with respect to calcification fronts. This means that even these cases included several with osteoid within normal limits. The evaluation of osteoid using toluidine blue or the Goldner method agreed fairly well throughout these studies although the measures were by no means always identical.

In the morphometric studies the osteoid-covered trabecular surface has been evaluated rather than the thickness of the osteoid seams. A product of the surface and the thickness would of course be the best measure of the total mass of osteoid. However, with very few exceptions, the osteoid was scarce and the seams fairly thin in the patients included in the present study. Therefore, the thickness of the osteoid and the total volume of osteoid in relation to total volume of bone will be a variable with such a large variation as to be almost useless. On the other hand, in patients with a large amount of osteoid such as some hemodialysis patients have, the total amount of osteoid would be a better parameter of bone disease than the surface.

Normative data (I)

Osteoid seams were very rare in the individuals chosen to represent the normal. There was a skewed distribution of the data and many samples did not contain any discernible osteoid at all. The Goldner method is probably the best for detecting even thin osteoid seams and therefore the staining method cannot explain the deviation from other investigators (Table 1, introduction). However, other workers also disagree with regard to the actual normal levels of osteoid in bone. The very low values observed in our study could lead to the suspicion that the selected cases did indeed deviate in a negative direction. However, very similar values were found in patients with back pain and in alcoholics without previous gastrectomy. Several explanations may be suggested for the low normative values in the present study:

- a. Some of the other authors use thicker sections which may show up as more osteoid.
- b. By counting selected areas in many sections from each sample it is possible to overestimate the actual amount of osteoid owing to the uneven distribution in bone - in the present study the entire area of one section was counted, a proceeding which does not allow for

selection of areas.

- c. There exists a very real difference so that residents of southern Sweden have less osteoid in their trabecular bone than other populations studied so far. This possibility cannot be entirely ruled out.

Point counting of the sample which gives an estimate of the per cent volume of osteoid is somewhat more consistent among the various studies on normative data (Table I, introduction), particularly when the poor precision of this method in patients with very little osteoid is taken into account.

Osteoporosis (II, III)

In the present study there was no significant difference between osteoporotic women and controls. The occurrence of osteoid was similarly rare in both groups and less than in all other sets of normative data presented in the past. Regardless of the absolute values, we must conclude that osteomalacia or increased abundance of osteoid as a component of the osteoporosis in women is extremely rare so that bone biopsy of a patient with signs of spinal osteoporosis only and without additional findings indicating osteomalacia is not worth the cost and effort invested or the discomfort to the patient.

An additional support of this concept of a poor relationship between the two conditions osteomalacia and osteoporosis are the findings on alkaline phosphatase in women with osteoporosis. In this study the alkaline phosphatase was related to a low bone mineral content rather than to an increased amount of osteoid. If alkaline phosphatase is interpreted as a sign of increased bone formation (Chalmers 1968) or high total turn-over then this is more closely related to the final outcome - decreased bone mineral mass - than to the presence of uncalcified bone matrix. Therefore our data do not support the hypothesis that the calcification process is impaired in osteoporosis. Treatment with calcium such as suggested by Lindahl (1960), Nordin (1964) and Bodfors et al. (1974) may therefore have its value by depressing the parathyroid activity and preventing resorption rather than improving the mineralization process.

Nutritional osteomalacia (I)

The relationship between uncalcified bone tissue and gastric surgery is fairly well established (Morgan et al. 1965). This fact is supported by our data in that patients who had undergone gastric resection

whether they were alcoholics or not had an approximately doubled amount of osteoid. However, only a few per cent had osteoid in an order of magnitude that would indicate true osteomalacia. The effects appear, in fact, to be marginal. Gastric resection due to peptic ulcer is today a rare procedure; in our biopsy patients it had been undertaken many years ago. Nevertheless, there were traces of impaired mineralization. Similar traces were found in the former concentration camp victims in spite of the fact that their hardships had taken place 30 years before the biopsy. Does starvation cause a permanent calcium absorption defect or does the osteoid in these cases represent bone tissue that cannot become mineralized in spite of an ample supply of calcium and vitamin D? The data of our study offer no answer to these questions.

Drug induced osteomalacia (IV, V)

Even if there are several reports on drug induced osteomalacia, the most recent and perhaps the most important being the relationship of osteomalacia and aluminum containing antacids, the epilepsy - anticonvulsant drug related osteomalacia is today the best documented. The increase in the amount of osteoid in the present study as well as in the data of Mosekilde and Melsen (1976, 1980) was, in most instances, relatively small and rarely reached a level consistent with the diagnosis of osteomalacia. Epilepsy in combination with anticonvulsant drugs appears to create a mixed skeletal disorder with a slightly reduced bone mineral content, an increased number of fractures, increased osteoclastic resorption and, finally, an increased amount of inactive osteoid. From the findings in our study we have suggested that these changes could be induced by interaction of the anticonvulsant drugs with the metabolic pathways of vitamin D and, possibly, by the relative physical inactivity of the institutionalized epileptics. Since the high fracture incidence creates great problems the morphometric findings are, in our opinion, sufficient evidence for the need of supplementary therapy.

Alcohol is a drug that would fail to be passed by the drug administrations in most countries. The most obvious complications would by no means be skeletal since the reduction of bone mass caused by alcohol takes decades to develop (Nilsson and Westlin 1973). The data of the present study do not support the hypothesis that the bone loss in alcoholics is due to dietary deficiency. The effect is probably related to an increased mobilization of calcium from bone through an

increased parathyroid activity and bone resorption which in the end will increase the risk of fracture.

Interpretations of increased osteoid

Osteoid seams are referred to by several investigators (Frost 1967, Sissons et al. 1967, Merz and Schenk 1970 and Bordier and Tun Chot 1972) as areas of bone formation. In spite of the fact that the turnover of bone is a constant process there was in many samples from particularly the normal controls and from women with osteoporosis very little osteoid if any. This may be due to a local variation and to the fact that with normal calcification the osteoid seam is of a very short duration and will become mineralized before it has attained any substantial width. If, on the other hand, the mineralization process is being delayed in spite of normal formation of bone matrix the osteoid seams will be more frequent and wider. Such changes can, for instance, be seen in the epileptics and do not necessarily indicate an increased bone turnover. In normal patients, without any signs of bone pathology, also, even if rarely, an increased abundance of osteoid may be observed usually now represented as thin seams. In such cases, possibly individuals or local areas of bone have been caught in the act of bone remodeling. Such incidences may be difficult to detect in normal individuals.

The data of the present study demonstrate that, even if osteomalacia in its clinical and/or morphological sense is fairly rare, an increased amount of osteoid is a fairly frequent finding in many groups of patients suspected for or suffering from skeletal disorders. This is true for well defined groups such as those patients who have undergone gastric surgery and for the less well defined groups such as those patients who have had multiple fractures or who suffer from miscellaneous symptoms that might be attributed to skeletal disorders. However, some groups such as those with backache as their only symptom or alcoholics who have not undergone gastric surgery do not significantly deviate and are in fact, as regards their amount of osteoid, almost identical with the controls.

GENERAL SUMMARY AND CONCLUSIONS

Bone biopsies were obtained from the iliac crests of 164 post-mortem cases and 252 patients referred owing to various clinical conditions. The biopsy technique of Burkhardt and the morphometric evaluation of Merz and Schenk were used and the following variables were estimated: Trabecular bone volume, per cent trabecular surface covered with osteoid, per cent active osteoid - calcification fronts - and the number of osteoclasts in relation to the surface of the section. Using the bone samples from the post-mortem cases as normative values, the amount of osteoid - active and inactive - was studied in various diagnostic groups.

The following conclusions could be drawn:

1. The Burkhardt instrument is a useful tool for obtaining good bone biopsies from the iliac crest.
2. The histological procedure and morphometric evaluation by Merz and Schenk are easy to handle once the method of hard tissue sectioning is mastered.
3. The working precision of estimation of osteoid is no better than 20 per cent but owing to the considerable inter-patient differences useful information can still be obtained.
4. Very little osteoid was found in individuals without any known or suspected metabolic bone disease - the normative data of the post-mortem cases in this study - considerably less than described by other investigators. It is most likely that deviations in the histological and morphometric techniques may explain this difference even if a real deviation may exist in the studied population.
5. Osteomalacia - increased amounts of osteoid - is rare in women with spinal osteoporosis.
6. The alkaline phosphatase which in women with osteoporosis is related to a low bone mineral content is unrelated to the amount of osteoid in these women.
7. Traces of impaired mineralization - osteoid seams in an increased abundance - were demonstrated in former concentration camp victims, possibly caused by a period of starvation in the past.

8. The amount of osteoid, in particular inactive osteoid, is increased in epileptic patients treated with anticonvulsant drugs.
9. Other findings in this group of patients are a noticeably high incidence of fractures and a low bone mineral content indicating that the finding of osteoid in such cases is evidence for the need of supplementary therapy.
10. In alcoholics there is no increase in the amount of osteoid, the mineralization of bone in these patients appearing to be undisturbed.
11. In alcoholics who also have undergone gastric resection, however, bone mineralization is disturbed as indicated by an increased amount of osteoid seams.
12. Although osteomalacia in its clinical and morphological sense is a rare condition, an increased amount of osteoid is a frequent finding in many groups of patients suspected of skeletal disorder.
13. On the other hand, in women with spinal osteoporosis without any other signs or symptoms of osteomalacia, increased osteoid is so rare that a bone biopsy is not justified.

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